

Biologic Antimicrobial Countermeasures

*“The enemy of my enemy
is my friend*”*

** usual caveats apply...*

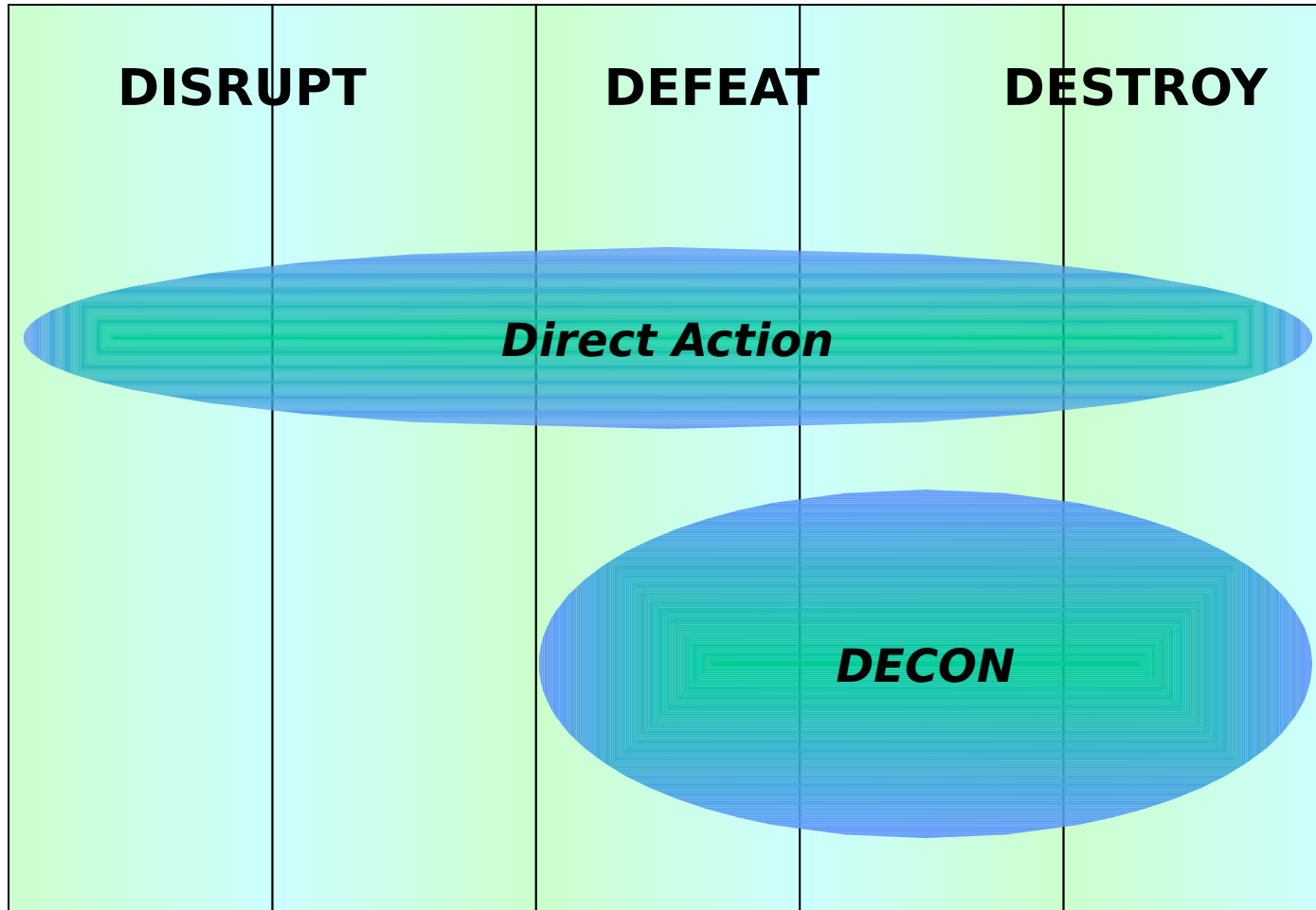
Devon Byrd
DTRA/ASX



Overview

- Bacteriophage are viruses that specifically destroy bacteria
- They use tricks shared by bacteria that kill other bacteria
- Most higher organisms have ways to kill bacteria
- These mechanisms are discoverable and exploitable
- In general, very efficient and man portable

Biologic Countermeasures Cut Across Agent Defeat Missions

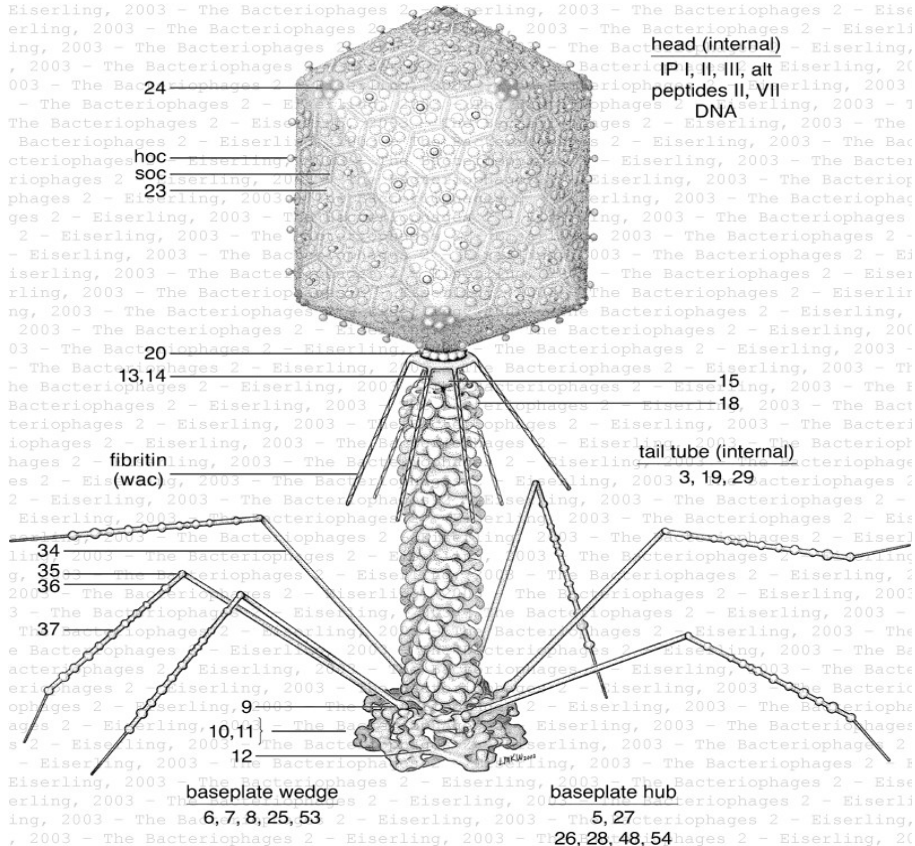


Making the World Safer

Bacteriophage (or phage for short) **kill bacteria**

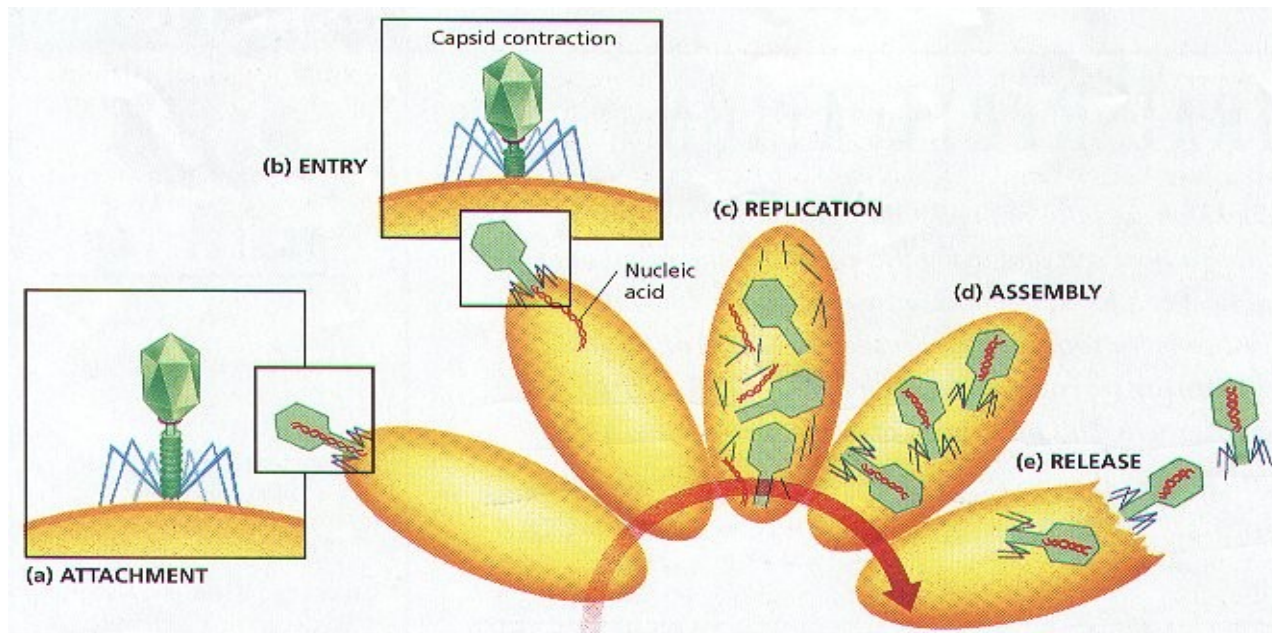


Generic Bacteriophage Structure



Making the World Safer

Lytic Phage Replication

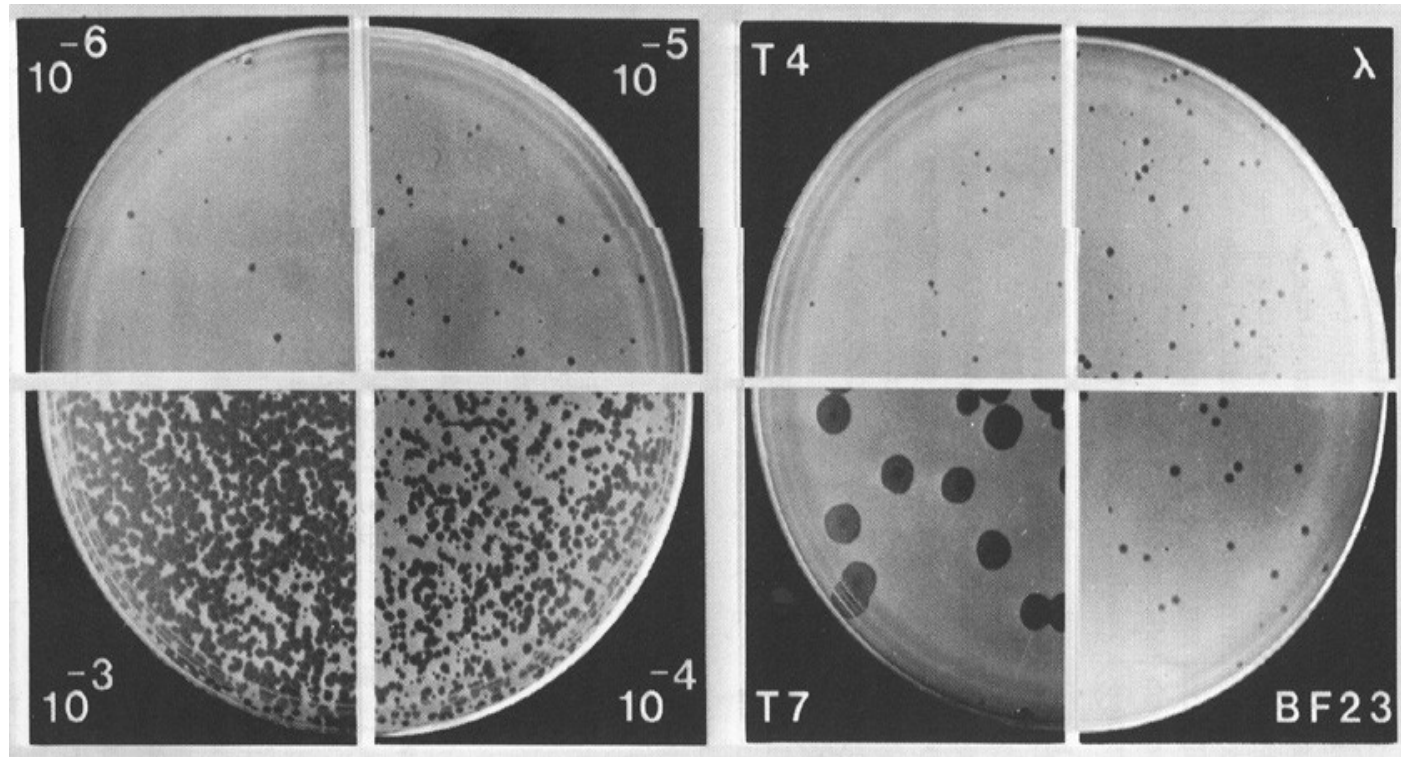


Lysis of bacterial cells by phage

(courtesy of Ry Young, TAMU)



As lytic phage propagate, bacteria are destroyed

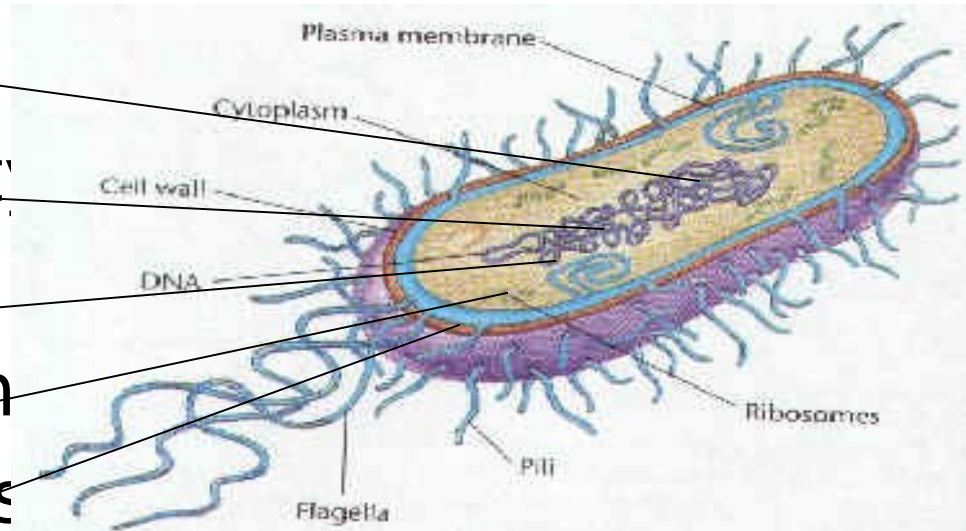


Bacteriophage target many aspects of host microbial metabolism-
Can be independently and/or synergistically exploited

Phage produce products that disrupt the following

bacterial systems:

- Genome integrity
- DNA replication
- Gene expression
- Protein synthesis
- Cellular integrity



Phage are a paradigm for biologically-based antimicrobial countermeasures

- Bacteria have been in a molecular arms race for billions of years
- They have thoroughly worked out the best ways to kill each other...
- ...as have other organisms (innate immunity)
- Very rich area for antimicrobial countermeasure R&D and acquisition

Categories of antimicrobial proteins

- Enzymatic

- Lysozymes
- B-glucosidases
- Nucleases
- Proteases

} **Bugs**

→ **Toxins and spores**

- Non-enzymatic

- Very effective on microbes (bacteria, viruses, fungi, etc.)
- Some evidence effective on spores
- Probably not useful for toxins
- Bacteriocins- produced by bacteria
- Antimicrobial peptides (AMPs)- produced by higher organisms



Making the World Safer

Examples of anti microbial agents that are produced by bacteria

Anti sigma factors

- Inactivate bacterial transcription factors

Toxin/antitoxin systems

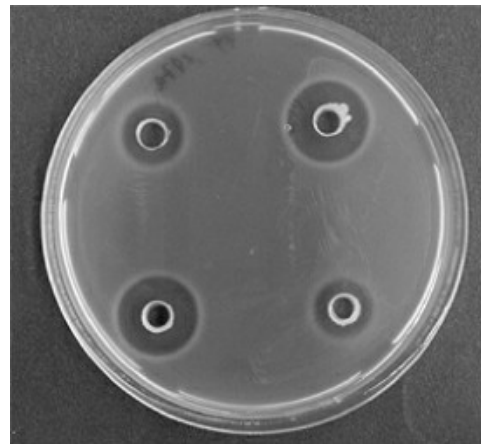
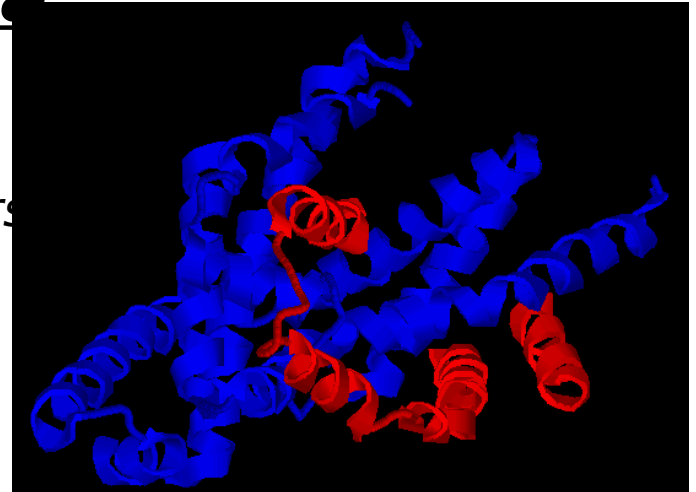
- Disrupt various metabolic components

Autolysins

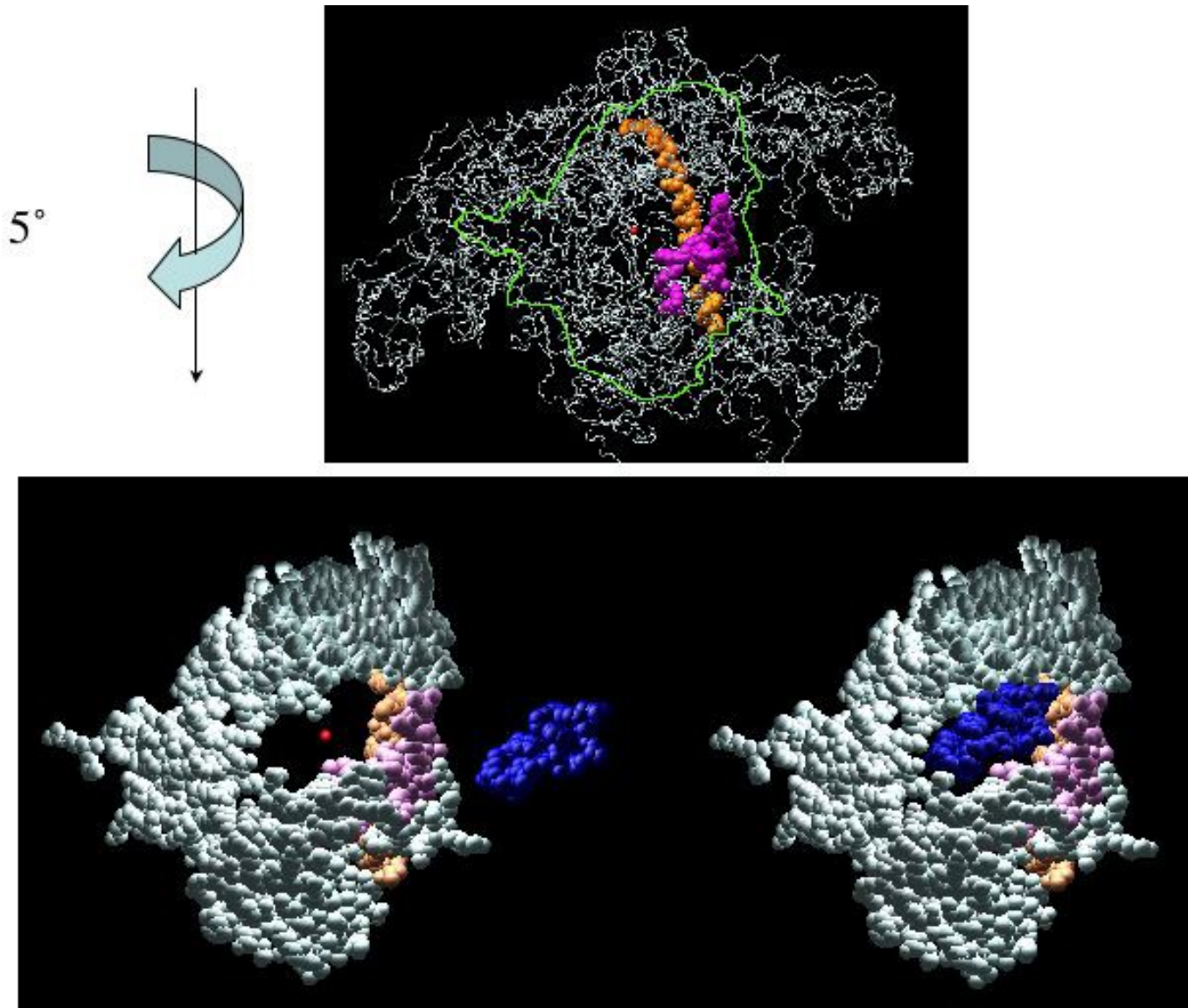
- Programmed cell death

Bacteriocins

- Cell lysis
- Replication
- Protein synthesis
- Gene expression



Interaction of MccJ25 with RNAP secondary channel: a cork in the bottle



Non-bacterial Sources

Amphibians

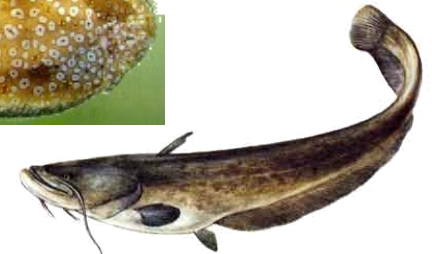


**Magainins
Bombinins
Citropins**



**Cecropins
Thanatin
Androctonin
Defensins**

Fish



**Pardaxin
Parasin
Hepcidin**

Nematodes



**ASABF
(*Ascaris suum* antibacterial factor)**



Making the World Safer

Location

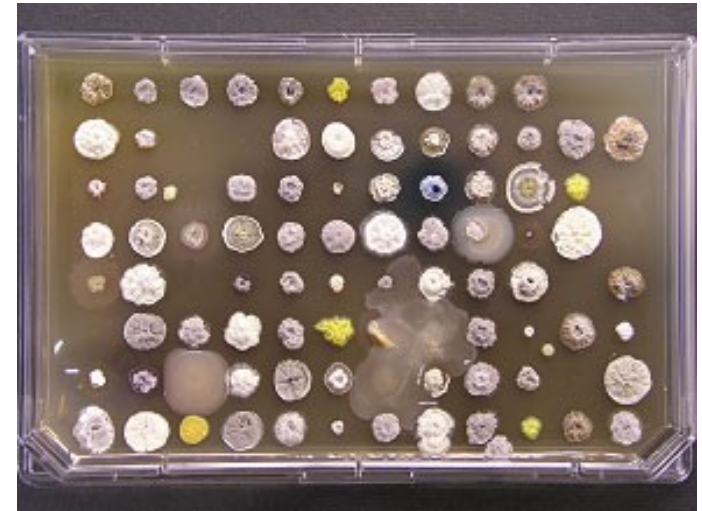
S



Making the World Safer

Bio-prospecting

- Go somewhere (hi/low, hot/cold, wet/dry... it doesn't matter- something will live there)
- Acquire indigenous knowledge (if possible)
- Recover specimens from various environments (soil, water, bugs, worms...)
- RTB specimens
- Analyze for useful properties/products



Potency of selected AMPs

<i>Antimicrobial Agent</i>	<i>Gram (+)</i>	<i>Gram (-)</i>	<i>Amount required to sterilize 1000 liters of culture*</i>	<i>Heat/Dessication Stable</i>
<i>RH5</i>	Y	Y	25 g (0.9 oz)	YES
<i>Thanatin</i>	Y	Y	25 g (0.9 oz)	YES
<i>CecropinB</i>	N	Y	6 g (.02 oz)	YES
<i>Hepcidin</i>	Y	Y	60 g (2.1 oz)	YES
<i>Magainin 2</i>	Y	Y	10 g (0.4 oz)	YES
<i>Temporin F</i>	Y	Y	14 g (0.5 oz)	YES
<i>Synthetic</i>	Y	Y	5 g (0.1 oz)	YES
<i>Phage</i>	Y	Y	1-1000 particles	NO

***NOTE: values are extrapolated from reported data**

Phage

- Tend to be specific for a given target agent
- Need specific knowledge of target in order to deliver appropriate phage
- ...or...
- Need to carry all possible phage for all possible target organisms
- No synergy when combined
- Essentially zero weight

Peptides

- More generic- a properly selected peptide will be effective against a fairly wide range of bacteria
- Exhibit synergistic effects when combined- dramatic increase in potency and target range
- Extremely light weight
- Generally heat and desiccation stable

Phage and peptides can be combined for maximum effect

Selected Web Resources for antimicrobial peptides

- <http://aps.unmc.edu/AP/main.php>
- <http://www.bbcm.units.it/~tossi/pag1.htm>
- <http://oma.terkko.helsinki.fi:8080/~SAPD/>



Making the World Safer

Conclusions

- Funding for mission-oriented, biologically-based antimicrobial/antitoxin R & D is needed
- Explore synergistic effects using mixed agent defeat components
- Work with subject matter experts to develop and optimize CONOPS
- Bring back specimens